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Perfluorochemicals and Human Semen Quality: The LIFE Study

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Abstract

Background: The relation between persistent environmental chemicals and semen quality is evolving, though limited data exist for men recruited from general populations.

Objectives: To assess the relation between perfluorinated chemicals (PFCs) and semen quality among 501 male partners of couples planning pregnancy.

Methods: Utilizing population-based sampling strategies, we recruited 501 couples discontinuing contraception from two U.S. geographic regions from 2005-2009. Baseline interviews and anthropometric assessments were conducted followed by blood collection for the quantification of 7 serum PFCs (perfluorosulfonates, perfluorocarboxylates and perfluorosulfonamides) using tandem mass spectrometry. Men collected a baseline semen sample and another approximately a month later. Semen samples were shipped with freezer packs, and analyses were performed on the day after collection. We used linear regression to estimate the difference in each semen parameter associated with a one unit increase in the natural log transformed PFC concentration after adjusting for confounders and modeling repeated semen samples. Sensitivity analyses included optimal Box-Cox transformation of semen quality endpoints.

Results: Six PFCs (2-N-methyl-perfluorooctane sulfonamide acetate [Me-PFOSA-AcOH], perfluorodecanoate [PFDeA], perfluorononanoate [PFNA], perfluorooctane sulfonamide [PFOSA], perfluorooctane sulfonate [PFOS], and perfluorooctanoate [PFOA]) were associated with 17 semen quality endpoints before Box Cox transformation. PFOSA was associated with smaller sperm head area and perimeter, lower percentage of DNA stainability, and a higher percentage of bicephalic and immature sperm. PFDeA, PFNA, PFOA, and PFOS were associated with a lower percentage of sperm with coiled tails.

Conclusions: Select PFCs were associated with certain semen endpoints, with the most significant associations observed for PFOSA though with results in varying directions.

Introduction

Perfluorochemicals (PFCs) are a group of synthetic chemicals that have been used in many consumer products (ATSDR 2009). The two highest production PFCs in the United States are perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), and are frequently detected in humans (ATSDR 2009; Kato et al. 2011). Other PFCs include perfluorohexane sulfonic acid (PFHxS), which is a member of the same chemical category as PFOS, and perfluorononanoic acid (PFNA), which is a member of the same chemical category as PFOA (U.S. EPA 2009). Chemicals within a given PFC chemical category share similar chemical structures making them stable and suitable for surface coating and protectant formulations for paper packaging products, carpets, leather products, and textiles that repel water, grease and soil among other uses (ATSDR 2009). Varying (in)direct sources of environmental exposure serve as routes for human exposure (Prevedouros et al. 2006) including ingestion of food and water, inhalation, and lactational transfer (Froome et al. 2009). Two recent studies pointed to food consumption as the primary pathway of exposure to PFOS and PFOA (Kelly et al. 2009; Trudel et al. 2008), with an estimated daily uptake from food estimated to be 2-3 ng/kg (Fromme et al. 2009).

Some PFCs remain in the environment and bioconcentrate in animals (Conder et al. 2008; Fromme et al. 2009; Kelly et al. 2007, 2009; Lau et al. 2007), given their long half-lives ranging from 3.5 to 7.3 years (Olsen et al. 2007). PFCs are not lipophilic but do bind to serum albumin (Han et al. 2003), which facilitates their measurement in serum indicative of long-term exposure (Fromme et al. 2009).

For the most part, well designed epidemiologic research focusing on environmentally relevant concentrations of PFCs and human fecundity, or the biologic capacity of men and women for reproduction (Buck Louis 2011a), has only recently begun. This gap is in contrast to an evolving body of experimental animal evidence suggestive of altered male fecundity, or decrements in testosterone and increased estradiol serum levels in exposed rats and lower serum testosterone concentrations and epididymal sperm counts in exposed mice (Biegel et al. 1995; Shi et al. 2007; Wan et al. 2011). However, not all animal studies have reported evidence of adverse effects (Luebker et al. 2005).

Equivocal results have emerged from three distinct samples of men in whom select PFCs were quantified in serum or plasma along with varying semen analyses, viz., men from the general Danish population (Joensen et al. 2009, 2013), male partners of pregnant women (Olmer Specht et al. 2012; Toft et al. 2012) or couples seeking infertility treatment (Raymer et al. 2012). Negative associations were observed for the highest relative to the lowest PFOA and PFOS concentrations and the median number of normal spermatozoa in the general Danish population (Joensen et al. 2009), as was an increasing percentage of sperm cell morphology defects in relation to serum PFOS concentrations among male partners of pregnant women from two European countries, but not amongst Inuit men, all of whom participated in the INUENDO Study (Toft et al. 2012). No associations were reported for serum PFOA, PFOS, PFHxS or PFNA and DNA damage or apoptotic sperm cells in men from the INUENDO Study (Olmer Specht et al. 2012). A recent cross-sectional study reported no significant associations of plasma and seminal PFOS and PFOA concentrations with reproductive hormones or select semen quality endpoints among 256 men attending infertility clinics (Raymer et al. 2012). Neither morphology nor DNA fragmentation was considered in this latter study. A study involving 247 young men being

considered for military service in Denmark reported only one statistically significant negative association between PFHpS and progressively motile sperm, though several negative relations were observed between PFOS and serum total and free testosterone, free androgen index and other hormonal ratios (i.e., testosterone/luteinizing hormone; testosterone/estradiol; free testosterone/luteinizing hormone; free androgen index to luteinizing hormone) (Joensen et al. 2013).

This existing body of evidence is largely limited to assessment of a few PFCs (viz., PFOA and PFOS) in male partners of pregnant women or of couples seeking infertility treatment. To our knowledge, there has been no attempt to assess PFCs in relation to a wide range of semen quality parameters among men from the general population, in particular in the United States, serving as the impetus for study.

Methods

Study design and cohort

The LIFE Study cohort was utilized for assessing 7 PFCs in relation to 35 semen quality parameters in an attempt to explore possible associations. Briefly, 501 couples discontinuing contraception for the purposes of becoming pregnant were recruited from 16 counties in Michigan and Texas. Given the absence of established population-based sampling frameworks for identifying couples planning pregnancy (Buck et al. 2004), we utilized a marketing database in Michigan and the fish/hunting license registry in Texas to ensure a sufficiently large denominator necessary for identifying couples planning pregnancy, who are estimated to comprise approximately 1% of the population (Buck et al. 2004; Slama et al. 2006). Forty-two percent of eligible couples enrolled in the study as fully described elsewhere (Buck Louis et al.

2011b). Inclusion criteria were minimal and male partners only needed to be 18 years of age, in a committed relationship, without medically confirmed infertility, and able to communicate in English or Spanish.

Data and biospecimen collection

Research assistants traveled to participants' homes for the collection of data and biospecimens. Specifically, males completed baseline interviews followed by a standardized anthropometric assessment for the determination of body mass index (BMI). Following completion of the interview and using blood collection equipment determined to be free of the environmental chemicals under study, 10 cc of blood was obtained and transported on ice to the laboratory for processing of which 2 ml of serum were used for the analysis of PFCs. Full human subjects approval was obtained from all participating institutions, and all participants provided written informed consent prior to enrollment into the study.

Men provided two semen samples for research purposes following enrollment approximately a month apart. Specimens were obtained via masturbation without the use of lubricants following a two-day abstinence period and using at home collection kits (Royster et al. 2000). A button thermometer was attached to glass collection jars to monitor temperature every half hour throughout the process to ensure specimen integrity. All specimens were found acceptable and used for analysis. Men were instructed to place a glass sperm migration straw (Vitrotubes #3520, VitroCom Inc., Mt. Lakes, NJ) containing hyaluronic acid and plugged at one end into the jar as an exploratory marker of sperm motility and viability at the time of collection, and to record time of last ejaculation and any spillage on labels. When the specimen was ready for shipment, men called a toll-free hotline to report sending their semen samples. Specimens were shipped in

insulated shipping containers containing ice packs via overnight carrier to the andrology laboratory, National Institute of Occupational Safety and Health located in Cincinnati, Ohio.

Semen analysis

Upon receipt, all semen samples were found to be within acceptable temperature limits and were used for analysis. Samples were then warmed to 37^{o} C with volume being measured to the nearest 0.1 cc. Using established laboratory protocols inclusive of an ongoing quality assurance and control procedures (American Andrology Association 1996), 35 semen parameters were assessed for the baseline semen sample including 5 general characteristics (volume, straw distance, sperm concentration, total count, hypo-osmotic swollen), 8 motility measures, 6 sperm head measures, 12 individual and 2 summary morphology measures, and 2 sperm chromatin stability measures. Specifically, sperm motility was assessed using the HTM-IVOS (Hamilton Thorne, Beverly, MA) computer assisted semen analysis system (CASA), and sperm concentration using the IVOS system and the IDENT™ stain. Microscope slides were prepared for sperm morphometry and morphology assessments, the latter performed by Fertility Solutions® (Cleveland, Ohio). Sperm viability was determined by hypo-osmotic swelling (HOS assay) (Schrader et al. 1990; Jevendran et al. 1992). Inclusion of the migration straw was done so that the lab could microscopically assess the distance the vanguard sperm traveled to the nearest mm, to inform about sperm motility at the time of collection, in light of using next day analysis (Turner and Schrader 1996). Although some sperm survive past 24 hours (Stovall et al. 1994) and refrigerated samples maintain sperm chromatin structure (Morris et al. 2003), our next day motility and straw endpoints are exploratory, given the absence of established validity for interpreting findings as with clinical semen analysis. Sperm morphometry was conducted using the IVOS METRIX system, and morphology was assessed using both traditional and strict

classifications (WHO 1992; Rothmann et al. 2013). An aliquot of whole semen was diluted in TNE buffer and frozen for the sperm chromatin stability assay (SCSA®) analysis (Evenson et al. 2002), which was conducted on a Coulter Epics Elite Flow Cytometer (SCSA diagnostics, Brookings, SD). The SCSA® assay measures sperm DNA damage, which is then quantified as the percentage of separated or damaged DNA (% DNA fragmentation index, DFI) and the percentage of highly immature sperm nuclei with abnormal proteins (% high stainability) (Evenson 2013). A DFI of 25% is associated with diminished fecundity and fertility (Spano et al. 2000), as is a high stainability ≈35% (Ménézo et al. 2007). The second semen sample was obtained to corroborate azoospermia observed in the first sample prior to informing the male who was then advised to seek clinical care. An abbreviated semen analysis was performed on the second sample (i.e., volume, concentration, next-day motility, and sperm head morphology).

Toxicologic analysis

All analyses were conducted by the Division of Laboratory Sciences in the National Center for Environmental Health, Centers for Disease Control and Prevention, using established protocols for the quantification of seven PFCs: 2-(N-ethyl-perfluorooctane sulfonamido) acetate (Et-PFOSA-AcOH), 2-(N-methyl-perfluorooctane sulfonamido) acetate (Me-PFOSA-AcOH), perfluorodecanoate (PFDeA), perfluorononanoate (PFNA), perfluorooctane sulfonamide (PFOSA), perfluorooctane sulfonate (PFOS), and perfluorooctanoate (PFOA). Quantification was performed using isotope dilution high performance liquid-chromatography-tandem mass spectrometry and established operating procedures (Kato et al. 2011; Kuklenyik et al. 2005). All PFCs are reported in ng/ml. We used machine observed concentrations without substituting concentrations below limits of detection (LOD) consistent with contemporary methods aimed at minimizing associated bias (Richardson and Ciampi 2003; Schisterman et al. 2006). Serum

cotinine was quantified (ng/ml) using liquid chromatography-isotope dilution tandem mass spectrometry (Bernert et al. 1997).

Statistical analysis

In the descriptive phase of analysis, we assessed geometric means and 95% confidence intervals (CIs) for PFCs by site using the nonparametric Wilcoxon test. In the analytic phase, we used linear mixed models to estimate the difference in each semen quality parameter associated with a one-unit change in the natural log-transformed after adding a value of 1 to each PFC concentration. This method accounts for the correlation stemming from the use of up to two semen samples per male participant for the select endpoints measured in both samples (i.e., volume, concentration, next-day motility, and sperm head morphology). Of note, 378/473 (80%) provided two semen samples. We ran separate models for each PFC and semen parameter, and estimated beta coefficients (β) and 95% confidence intervals (CIs) for each model. Specifically, beta coefficients denoted the difference in each semen outcome per unit increase in each PFC. We adjusted *a priori* for age (years), body mass index (weight in kg/ height in m^2), smoking (serum cotinine >40.35 ng/ml or active smoking), abstinence time (days), sample age (hours), and study site (Carlsen et al. 2004; Jeemon et al. 2010; Jensen et al. 1998; Liu et al. 2011; Ramlau-Hansen et al. 2007; Sadeu et al. 2010; Schmid et al. 2013). We conducted sensitivity analyses using Box-Cox analysis to determine the optimal transformation for each semen variable. We found that semen endpoints required natural logarithm (n=14), cubic root (n=6) or no (n=14) transformation using the Shapiro-Wilk W statistic to assess all semen quality endpoints (Handelsman 2002). We also visually assessed the residual plots to affirm normality assumptions. Consistent with the exploratory nature of this work in light of limited data, we did not adjust for multiple comparisons. P-values <0.05 were considered statistically significance.

Results

A serum and at least one semen sample were available for 462 (92%) men; 11 men were missing serum, 26 semen and 2 both. There were no statistically significant differences in PFC concentrations between men who did or did not provide semen samples, except for a higher PFNA concentration in men without than with a semen sample (1.82; 95% CI: 1.52, 2.18 and 1.50; 95% CI: 1.43, 1.58, respectively) (see Supplemental Material, Table S1). The study cohort comprised mostly white non-Hispanic college educated men with a mean age 31.8 ± 4.9 years and a mean BMI 29.8 ± 5.6, with no significant differences by enrollment site (Table 1). Many (57%) men had previously fathered a pregnancy and few (17%) were current smokers. Mean (±SD) abstinence times for samples one and two were 4.0 (±4.5) and 4.3 (±5.6) days, respectively. Only 2 (0.4%) men reported an abstinence time <2 days for the initial sample, as did 10 (2.7%) men providing a second sample.

Table 2 presents the distributions for the seven PFCs by research site and reflects that most chemicals were readily detected in men's serum with the exception of Et-PFOSA-AcOH and PFOSA, where 97% and 84% of concentrations were <LOD, respectively. No statistically significant differences in PFC concentrations were observed between men who did or did not provide a semen sample, except for a higher PFNA concentration in men without than with a semen sample (1.82; 95% CI: 1.52, 2.18 and 1.50; 95% CI: 1.43, 1.58, respectively). Correlation coefficients between PFCs were low (range 0.02-0.6), though higher for PFNA and PFDeA (r=0.8), PFNA and PFOS (r=0.7) and PFOS and PFDeA (r=0.7).

When modeling each PFC and semen parameter individually, several significant associations were observed, some of which but not all were suggestive of diminished semen quality (Table 3).

Et-PFOSA-AcOH was the only PFC that was not associated with any semen quality parameter, but data for this compound are difficult to interpret given that 97% of observations were <LOD. Three semen quality endpoints were associated with two or more PFCs: 1) a reduction in the percentage of sperm with coiled tail with PFDeA, PFNA, PFOA, and PFOS; 2) a reduction in the percentage of sperm with high DNA stainability with MePFOSA-AcOH and PFOSA; and 3) an increase in the number of immature sperm with MePFOSA-AcOH and PFOSA in the primary analysis or without Box-Cox transformation. Other semen quality parameters were significantly associated with individual PFCs, but without a clear pattern as evident by the varying directions.

PFOSA, MePFOSA-AcOH and PFOA were the PFCs most often observed to be associated with semen quality endpoints, i.e., 5, 3 and 2 separate associations, respectively. Specifically, a 1-unit increase in log transformed PFOSA was associated with smaller sperm head area (β=-2.295; 95% CI: -4.052, -0.538), smaller sperm perimeter (β=-1.252; 95% CI: -2.276, -0.228), lower percentage of sperm with high DNA stainability (β=-15.153; 95% CI: -26.559, -3.747), higher percentage of bicephalic sperm (β=4.127; 95% CI: 0.149, 8.105), and higher numbers of immature sperm (β=90.881; 95% CI: 51.266, 130.496). Me-PFOSA-AcOH was associated with a higher percentage of sperm with neck/midpiece abnormalities (β=5.011; 95% CI: 0.724, 9.298), higher numbers of immature sperm (β=18.719; 95% CI: 11.611, 25.827), and a lower percentage with high DNA stainability (β=-2.552; 95% CI: 04.665, -0.438). In sensitivity analyses 16/17 significant ($p < 0.05$) associations were observed, of which 15 were observed in the primary analysis, i.e., specifically, volume was not significant in the sensitivity analysis. Complete sensitivity results are provided in Supplemental Material, Table S2.

Discussion

Our findings suggest that select PFCs at environmentally relevant concentrations may be adversely associated with semen quality with the exception of Et-PFOSA-AcOH, possibly given that only 3% of concentrations were >LOD. Associations suggestive of diminished semen quality included differences in sperm head (increased bicephalic) and morphology (increased immature sperm). Interpretation of these findings is uncertain, given the lack of well-established clinical norms for many individual parameters and reliance on next day semen analysis and possible spurious associations. Morphologic sperm characteristics including those involving the tail may be informative about underlying mechanisms during the maturation process and eventual fertility, particularly in the context of other biochemical markers (Durutovic et al. 2013). Also, it should be noted that each of these PFCs was associated with other semen characteristics underscoring their varying patterns. PFOSA, a precursor to PFOS, was significantly associated with the most semen parameters, including an increased percentage of bicephalic and number of immature sperm suggestive of diminished semen quality. However, we recognize the absence of established levels between normal sperm head morphology and genetic quality of spermatozoa (Ryu et al. 2001; Simon and Lewis 2011), limiting further speculation regarding our findings. We did not observe evidence of sperm DNA damage on the basis of SCSA® findings, given a lower percentage of damaged sperm irrespective of PFC. It is important to note that only 16% of PFOSA concentrations were above the LOD, though quantification of PFCs was blinded to semen quality. The low prevalence of such exposure with most of the measured concentrations largely at the LOD is consistent with its ceased U.S. production. The lack of consistent associations with motility other than two positive associations for percent motility and straw

distance may reflect our reliance on next-day analysis, which provides only an exploratory assessment of viability and motility at the timing of collection absent validation methods.

Our findings suggesting a negative association between select PFCs and sperm morphology are somewhat consistent with those reported for male partners of pregnant women seeking prenatal care (Toft et al. 2012), where a 35% reduction in the proportion of morphologically normal sperm was observed for men in the highest relative to the lowest tertile of PFOS. Given that tests for assessing sperm DNA are not equivalent, we were unable to assess earlier findings such as an increased percentage of TUNEL-positive sperm cells for serum PFOA concentrations as reported in 2/3 subgroups of male participants in the IUENDO Cohort, as we utilized the SCSA® method. We also corroborated the absence of an association between PFCs and the percentage of DNA fragmentation when using SCSA techniques (Olmer Specht et al. 2012). Of note was our observation of a negative association between Me-PFOSA-AcOH and PFOSA and the percentage of high DNA stainability, suggesting fewer sperm with immature chromatin. The absence of previous research focusing on this outcome precludes a more complete interpretation of this finding.

Median concentrations of PFCs for men participating in the LIFE Study were comparable to five earlier papers (see Supplemental Material, Table S3), despite differences in sampling frameworks ranging from medical clinics (Olmer Specht et al. 2012; Raymer et al., 2012; Toft et al. 2012) to the general population (Joensen et al. 2009, 2013). In addition to recruiting men from targeted geographic areas who were not seeking medical care, our findings are further strengthened by the availability of both PFC and semen quality data for 92% of the cohort, the analysis of all semen samples provided by men, and our direct measurement of BMI and serum cotinine. PFC concentrations of participants in the LIFE Study were comparable to those

reported by NHANES despite the older age range of its participants (CDC 2009). However, geometric mean PFOS concentrations were higher in the LIFE Study than NHANES (20.52; 95% CI: 19.47, 21.63 and 16.3; 95% CI: 15.0, 17.70, respectively).

Still important limitations need to be considered in the interpretation of our results, most notably the use of a next-day semen analysis. There has been limited study of home versus clinical semen collection underscoring the exploratory nature of next day analysis. Our motility findings have uncertain meaning and cannot be directly compared with clinical semen analysis. Previous researchers successfully utilized home semen collection for the assessment of environmental exposures, but they did not report motility findings (Luben et al. 2007; Olshan et al. 2007). The authors did, however, note that all returned samples retained motile and viable sperm. Our straw measure was used as a similar global measure. A second limitation is the timing of exposure relative to semen collection, an issue relevant for all past research as well, which makes it difficult to identify the sensitive window. While the long half-lives of PFCs most likely precede the relevant sensitive window (≈72 days) for spermatogenesis and hormonal milieu for the analysis of our semen samples, we recognize that we cannot rule out possible in utero exposures that result in epigenetic spermatozoa defects (Aitken 2010). However, we are unable to address the exact timing of exposure relevant for semen quality such as the *in utero* window as recently reported (Vested et al. 2013). A third limitation is the absence of any reproductive hormone measurements, particularly given earlier reports of both a negative relation between serum PFOS and testosterone (Joensen et al. 2013) and a positive one for plasma PFOA and PFOS (Raymer et al. 2012). Lastly, we cannot rule out chance findings given our exploratory analysis that comprised 245 comparisons, of which 7% were found significant at $p < 0.05$.

Our findings may have potential implications for male reproductive health or couple fecundity. While semen analysis provides useful information on sperm production, motility, viability, genital tract patency, accessory gland function, and ejaculation capability, its predictive value for fertility remains limited (ASRM 2013; Niederberger 2011) underscoring the need for continued investigation of fecundity biomarkers suitable for population health research.

The mechanisms through which PFC exposure may affect semen quality remain unknown, though several posited explanations have been suggested including their estrogenic-like properties (Liu et al. 2007), and ability to alter hormonal milieu (Damgaard et al. 2002), lipid metabolism (Kennedy et al. 2004), inflammatory processes and responses (Corsini et al. 2012), and reactive oxygen species (Mathur et al. 2008). Irrespective of mechanism(s), our findings support continual efforts aimed at elucidating environmental impacts on semen quality, particularly given increasing global concern about declining male fecundity (Pflieger-Bruss et al. 2004; Priskorn et al. 2012). Despite these concerns, limited research has been conducted to determine the impact of environmental chemicals on human semen quality, resulting in continued reliance on animal findings (Phillips and Tanphaichitr 2008). The highly timed and interrelated nature of spermatogenesis, whereby immature spermatogonia develop into mature spermatids for their eventual release and continued maturation through the epididymis, underscores their vulnerability throughout this sensitive window in relation to environmental influences.

Conclusions

Select PFCs at environmentally relevant concentrations were associated with differences in sperm head, morphology and DNA characteristics, including differences indicative of higher and

lower semen quality. These exploratory findings are suggestive of some deleterious differences in sperm morphology (e.g., immature, bicephalic), but await corroboration. Follow up investigation of the impact of semen changes on male reproductive health or couple fecundity is needed including in-depth semen analyses.

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Table 1. Description of male partners by study site, LIFE Study.

Characteristic	Michigan (n=96)	Texas (n=366)	Total (n=462)
Nonwhite race/ethnicity	9 (9)	42 (12)	51 (11)
≤ High school education	8 (8)	28 (8)	36 (8)
No health insurance	10 (10)	28 (8)	38 (8)
Never fathered a pregnancy	47 (49)	193 (53)	240 (52)
Current smoker (cotinine > 40.35 ng/ml)	18 (19)	66 (18)	84 (18)
Age (in years)	32.1 ± 4.5	31.7 ± 4.9	31.8 ± 4.9
Body mass index (kg/m^2)	29.7 ± 5.4	29.9 ± 5.8	29.8 ± 5.7
Abstinence (days)	4.4 ± 4.0	4.0 ± 5.2	4.1 ± 5.0
Sample age (hours)	28.5 ± 10.0	27.8 ± 8.2	28.0 ± 8.6

Data are n (%) or mean ± SD.

NOTE: None of the above differences were statistically significant as all *p*-values (from chi-square test for categorical and Wilcoxon rank sum test for continuous characteristics) comparing the two sites were > 0.05.

Table 2. Distribution of serum perfluorochemical concentrations by availability of semen samples, LIFE Study.

PFC (ng/ml)	% < LOD	Michigan site (n=96) Geometric mean (95% CI)	Michigan site (n=96) Median (IQR)	Texas site (n=366) Geometric mean (95% CI)	Texas site (n=366) Median (IQR)
Et-PFOSA-AcOH	97	0.12 (0.10, 0.14)	0 (0, 0.1)	0.12 (0.10, 0.13)	0 (0, 0)
Me-PFOSA-AcOH	22	0.47 (0.40, 0.54)	0.4 (0.3, 0.7)	0.29 (0.26, 0.31)	0.25 (0.1, 0.5)
PFDeA	5	0.31 (0.28, 0.35)	0.3 (0.2, 0.4)	0.47 (0.45, 0.50)	0.5 (0.3, 0.6)
PFNA	1	0.96 (0.84, 1.11)	1.0 (0.75, 1.35)	1.68 (1.61, 1.76)	1.65 (1.2, 2.2)
PFOA	< 1	4.29 (3.86, 4.77)	4.6 (3.0, 6.05)	5.09 (4.86, 5.33)	5.3 (4.1, 6.6)
PFOS	< 1	17.39 (14.94, 20.24)	19.15 (14.65, 25.7)	21.23 (20.07, 22.46)	21.6 (15.8, 29.9)
PFOSA	84	0.13 (0.11, 0.15)	0 (0, 0.1)	0.11 (0.10, 0.12)	0 (0, 0)

NOTE: *P*-values reflect the comparison of means by site. All differences were significant ($p \leq 0.02$). Thirty-nine men were excluded given the absence of PFC measurements (n=13) or semen samples (n=2).

CI, denoted confidence interval; IQR, denotes interquartile range; LOD, denotes < limits of detection.

Table 3. Estimated difference in semen quality parameters associated with a 1-unit increase in natural log-transformed serum perfluorochemical concentrations, LIFE Study.

Semen parameter	**Et-PFOSA-AcOH** β (CI 95%)	**Me-PFOSA-AcOH** β (CI 95%)	**PFDeA** β (CI 95%)	**PFNA** β (CI 95%)	**PFOA** β (CI 95%)	**PFOS** β (CI 95%)	**PFOSA** β (CI 95%)
General characteristics[a]							
Volume (mL)	-0.452 (-1.837, 0.933)	-0.550 (-1.118, 0.018)	0.630 (-0.195, 1.455)	0.093 (-0.388, 0.574)	-0.092 (-0.457, 0.273)	0.058 (-0.197, 0.312)	-1.433 (-4.626, 1.760)
Viability (%)[a]	3.154 (-5.212, 11.520)	-0.342 (-3.932, 3.248)	1.612 (-3.451, 6.675)	1.564 (-1.378, 4.506)	-0.777 (-3.016, 1.463)	-0.056 (-1.621, 1.509)	-5.922 (-25.61, 13.767)
Total count (concentration x10^6/mL)	18.873 (-143.522, 181.267)	-9.951 (-77.147, 57.246)	81.003 (-16.059, 178.065)	45.125 (-11.347, 101.598)	0.679 (-42.300, 43.658)	9.741 (-20.243, 39.724)	130.776 (-246.228, 507.780)
Sperm concentration (x10^6/mL)	14.848 (-34.366, 64.062)	8.200 (-12.056, 28.456)	-1.063 (-30.457, 28.332)	5.218 (-11.870, 22.306)	0.388 (-12.581, 13.357)	0.025 (-9.018, 9.068)	47.187 (-66.294, 160.668)
Sperm motility[a]							
Average path velocity (μm/sec)	2.153 (-8.455, 12.761)	-0.106 (-4.641, 4.430)	3.761 (-2.637, 10.158)	2.293 (-1.426, 6.013)	2.223 (-0.603, 5.050)	0.378 (-1.598, 2.355)	9.433 (-15.420, 34.287)
Straight line velocity (μm/sec)	2.380 (-6.296, 11.056)	-0.145 (-3.861, 3.570)	2.947 (-2.293, 8.186)	2.118 (-0.927, 5.162)	1.614 (-0.702, 3.930)	0.646 (-0.972, 2.264)	1.257 (-19.117, 21.632)
Curvilinear velocity (μm/sec)	0.151 (-18.037, 18.340)	-0.664 (-8.453, 7.125)	4.773 (-6.216, 15.762)	3.139 (-3.25, 9.528)	4.982* (0.137, 9.827)	0.293 (-3.101, 3.688)	16.779 (-25.918, 59.475)
Amplitude head displacement (μm)	-0.372 (-1.523, 0.780)	-0.328 (-0.825, 0.168)	0.419 (-0.281, 1.119)	0.176 (-0.232, 0.583)	0.165 (-0.145, 0.475)	-0.086 (-0.303, 0.131)	-0.354 (-3.089, 2.380)
Beat cross frequency (Hz)	2.662 (-3.234, 8.559)	-0.735 (-3.289, 1.819)	0.608 (-2.987, 4.203)	0.542 (-1.549, 2.633)	1.374 (-0.213, 2.961)	-0.136 (-1.249, 0.976)	-3.545 (-17.583, 10.492)
Straightness (%)	7.705 (-8.556, 23.966)	0.117 (-6.898, 7.132)	6.975 (-2.888, 16.839)	4.019 (-1.718, 9.756)	3.736 (-0.627, 8.099)	1.254 (-1.800, 4.308)	13.216 (-25.281, 51.714)
Linearity (%)	7.164 (-3.424, 17.753)	0.420 (-4.168, 5.009)	5.507 (-0.932, 11.946)	3.021 (-0.725, 6.768)	1.570 (-1.286, 4.427)	1.116 (-0.881, 3.112)	11.462 (-13.728, 36.653)
Percent motility (%)	5.915 (-4.500, 16.331)	0.676 (-3.732, 5.085)	3.672 (-2.564, 9.908)	2.516 (-1.108, 6.139)	1.437 (-1.319, 4.192)	1.556 (-0.361, 3.473)	-4.190 (-28.297, 19.918)
Sperm head[a]							
Length (μm)	-0.145 (-0.396, 0.105)	-0.029 (-0.134, 0.077)	-0.155* (-0.304, -0.006)	-0.064 (-0.151, 0.023)	-0.037 (-0.103, 0.029)	-0.029 (-0.075, 0.017)	-0.509 (-1.085, 0.066)
Area (μm^2)	-0.335 (-1.096, 0.427)	-0.150 (-0.473, 0.174)	-0.271 (-0.728, 0.186)	-0.201 (-0.467, 0.064)	-0.156 (-0.358, 0.046)	-0.132 (-0.272, 0.009)	-2.295* (-4.052, -0.538)
Width (μm)	0.006 (-0.151, 0.163)	-0.023 (-0.090, 0.043)	0.017 (-0.077, 0.111)	-0.023 (-0.078, 0.031)	-0.026 (-0.068, 0.015)	-0.024 (-0.053, 0.005)	-0.210 (-0.571, 0.152)

Semen parameter	**Et-PFOSA-AcOH** β (CI 95%)	**Me-PFOSA-AcOH** β (CI 95%)	**PFDeA** β (CI 95%)	**PFNA** β (CI 95%)	**PFOA** β (CI 95%)	**PFOS** β (CI 95%)	**PFOSA** β (CI 95%)
Perimeter (μm)	-0.249 (-0.695, 0.197)	-0.068 (-0.256, 0.121)	-0.227 (-0.493, 0.039)	-0.118 (-0.273, 0.037)	-0.066 (-0.184, 0.052)	-0.066 (-0.148, 0.016)	-1.252* (-2.276, -0.228)
Elongation factor (%)	1.476 (-3.295, 6.247)	-0.054 (-2.051, 1.942)	2.122 (-0.706, 4.949)	0.294 (-1.355, 1.942)	-0.203 (-1.454, 1.047)	-0.191 (-1.061, 0.679)	3.121 (-7.753, 13.995)
Acrosome area of head (%)	2.457 (-1.821, 6.735)	1.140 (-0.665, 2.945)	1.367 (-1.190, 3.924)	1.405 (-0.079, 2.889)	1.304* (0.180, 2.428)	0.288 (-0.500, 1.075)	9.785 (-0.047, 19.617)
Straw							
Distance (mm)[b]	-1.439 (-7.612, 4.734)	0.648 (-2.226, 3.522)	2.727 (-1.166, 6.621)	1.742 (-0.536, 4.019)	-0.001 (-1.674, 1.672)	1.231* (0.089, 2.372)	13.890 (-2.427, 30.206)
Morphology[b]							
Strict criteria (%)	4.816 (-4.735, 14.367)	-0.385 (-4.669, 3.900)	4.914 (-0.829, 10.658)	3.897* (0.564, 7.231)	1.916 (-0.561, 4.392)	1.720 (-0.012, 3.452)	18.709 (-4.918, 42.335)
WHO normal (%)	6.096 (-5.728, 17.920)	-0.432 (-5.737, 4.873)	5.799 (-1.313, 12.912)	3.968 (-0.168, 8.104)	1.726 (-1.345, 4.796)	1.835 (-0.312, 3.982)	25.417 (-3.815, 54.648)
Amorphous (%)	-4.101 (-14.484, 6.281)	0.981 (-3.674, 5.635)	-0.435 (-6.698, 5.827)	-1.437 (-5.080, 2.206)	-0.372 (-3.070, 2.327)	-0.259 (-2.150, 1.631)	-10.054 (-35.783, 15.674)
Round (%)	-1.309 (-2.832, 0.213)	-0.056 (-0.740, 0.629)	0.365 (-0.555, 1.285)	0.311 (-0.224, 0.846)	-0.020 (-0.416, 0.377)	0.162 (-0.116, 0.440)	-1.074 (-4.858, 2.711)
Pyriform (%)	-0.248 (-6.234, 5.739)	-0.126 (-2.809, 2.556)	-1.605 (-5.21, 2.000)	-0.012 (-2.113, 2.089)	-0.459 (-2.013, 1.096)	-0.632 (-1.719, 0.456)	-1.376 (-16.212, 13.459)
Bicephalic (%)	-0.186 (-1.800, 1.427)	0.283 (-0.439, 1.006)	0.658 (-0.312, 1.629)	0.169 (-0.397, 0.735)	0.325 (-0.093, 0.743)	0.205 (-0.088, 0.498)	4.127* (0.149, 8.105)
Taper (%)	-1.337 (-3.844, 1.170)	-0.623 (-1.746, 0.501)	-0.717 (-2.228, 0.795)	-0.051 (-0.932, 0.830)	0.117 (-0.535, 0.769)	0.037 (-0.42, 0.494)	-6.179 (-12.371, 0.014)
Megalo head (%)	-0.732 (-2.528, 1.064)	0.088 (-0.717, 0.893)	-0.632 (-1.713, 0.450)	-0.355 (-0.985, 0.275)	-0.392 (-0.857, 0.073)	-0.257 (-0.584, 0.069)	-1.085 (-5.537, 3.367)
Micro head (%)	-0.743 (-1.865, 0.378)	-0.171 (-0.675, 0.332)	0.114 (-0.563, 0.792)	-0.019 (-0.413, 0.375)	-0.214 (-0.505, 0.077)	-0.084 (-0.288, 0.120)	0.555 (-2.23, 3.340)
Neck or midpiece abnormalities (%)	-5.766 (-15.38, 3.848)	5.011* (0.724, 9.298)	-2.624 (-8.423, 3.175)	-2.462 (-5.833, 0.909)	-1.845 (-4.340, 0.650)	-0.512 (-2.264, 1.240)	-5.048 (-28.909, 18.812)
Coiled tail (%)	-6.898 (-17.585, 3.789)	0.099 (-4.699, 4.898)	-7.603* (-14.014, -1.193)	-4.030* (-7.766, -0.293)	-2.768* (-5.536, 0.000)	-2.274* (-4.209, -0.338)	-9.128 (-35.650, 17.395)
Other tail abnormalities (%)	-0.451 (-4.453, 3.551)	1.440 (-0.348, 3.227)	-0.354 (-2.766, 2.057)	-0.519 (-1.922, 0.885)	-0.112 (-1.151, 0.928)	-0.327 (-1.054, 0.401)	-3.089 (-13.002, 6.824)
Cytoplasmic droplet (%)	-0.949 (-6.000, 4.103)	-1.825 (-4.082, 0.431)	-0.172 (-3.217, 2.873)	0.123 (-1.65, 1.896)	-0.348 (-1.66, 0.963)	-0.407 (-1.326, 0.511)	-9.053 (-21.541, 3.436)
Immature sperm (#)	-4.963 (-21.346, 11.419)	18.719** (11.611, 25.827)	3.629 (-6.243, 13.501)	2.610 (-3.135, 8.355)	-0.411 (-4.668, 3.846)	2.095 (-0.880, 5.070)	90.881** (51.266, 130.496)

Semen parameter	Et-PFOSA-AcOH β (CI 95%)	Me-PFOSA-AcOH β (CI 95%)	PFDeA β (CI 95%)	PFNA β (CI 95%)	PFOA β (CI 95%)	PFOS β (CI 95%)	PFOSA β (CI 95%)
Sperm chromatin stability[b]							
DNA fragmentation index (%)	-2.634 (-11.979, 6.710)	1.234 (-2.859, 5.328)	-4.040 (-9.793, 1.712)	-2.878 (-6.155, 0.400)	-0.713 (-3.184, 1.757)	-0.914 (-2.678, 0.849)	-0.899 (-23.035, 21.236)
High DNA stainability (%)	-0.539 (-5.395, 4.317)	-2.552* (-4.665, -0.438)	-0.929 (-3.923, 2.065)	-0.687 (-2.395, 1.020)	-0.115 (-1.399, 1.169)	-0.423 (-1.340, 0.493)	-15.153** (-26.559, -3.747)

NOTE: Analysis includes 5 azoospermic men but excludes 39 men missing either PFCs (n=11), semen samples (n=26) or both (n=2). Fixed and mixed effects models were used for the analysis of semen parameters with one and two measurements, respectively. PFC concentrations were natural log transformed and adjusted for age (years), BMI (kg/m^2), serum cotinine (active smoking yes/no), abstinence (# days), sample age (# hours), and research site (Texas/Michigan).

[a]Assessed in both semen samples. [b]Assessed only in the baseline semen sample.

*$p<0.05$, **$p<0.01$.

β, beta coefficient; CI, confidence interval.